tissues that contain cells expressing selectin ligands, including secondary lymphoid tissues and other tissues. Therefore, the present invention enables one of skill in the art to improve upon current immunization techniques by genetically modifying dendritic cells to express the binding portions of the selectins necessary to target the dendritic cells to tissues that express the corresponding ligands.

All of the foregoing principles are presented in Applicants' specification, along with additional disclosures regarding the choice of selectin molecules that can be used in the claimed invention. These include L-selectin, E-selectin, P-selectin, including non-cleavable forms of L-, E- and P- selectins, and chimeric selectin molecules, e.g. the E/L-selectin disclosed in the working examples (see page 7, lines 23-30).

Regarding the Examiner's assertions of a lack of enablement for certain aspects of the invention made on page 3, Applicants note that the claimed invention is not as broad as indicated by the Examiner. For example, the Examiner asserted that the specification was not enabling for "delivering recombinant dendritic cells to any tissue in a mammal by transfecting said cell with any portion of L, E, or P selectin." The claimed invention does not recite that dendritic cells (DCs) are delivered to any tissue; claims 1, 14 and 21 specify secondary lymphoid tissue and claims 5, 18 and 25 specify that the dendritic cells are delivered to "non-lymphoid tissue of a subject where selectin ligands are expressed on endothelial cells," i.e., only tissues containing endothelial cells that express selectin ligands. Similarly, the claimed invention recites specific portions of selectin ("selectin polypeptide comprising an endothelial selectin ligand binding portion of a selectin"), not any portion as stated by the Examiner. Because this specific portion of selectin is recited, the methods and compositions operate through binding of the DCs to tissues that express selectin ligand. Therefore, the Examiner's use of the word "any" in connection with tissues, portions of selectins, and so forth would appear to be misplaced in view of the claim limitations and the description in the specification.

The examiner also stated that the specification "speculates" that homing of DCs will have an increased therapeutic effect on infections and cancer (Office Action at page 3). Applicants did not speculate on the effects of DC targeting to selectin ligand-expressing tissues, but rather stated that "the invention disclosed herein provides the unexpected result that augmentation of selectin polypeptides on the surface of cultured dendritic cells can alter the ability of the DCs to enter peripheral lymph nodes, thereby enhancing antigen presentation." (Page 9, lines 16-19). This statement was made in view of the well known antigen presentation properties of DCs. As

antigen presentation to naïve T cells in lymph nodes is accomplished efficiently by dendritic cells, enhanced antigen presentation would advantageously affect immune responses against antigens presented by DCs.

Regarding the statement made by the Examiner that one of ordinary skill in the art would not have predicted that DCs could be transfected to express sufficient levels of L-selectin (see page 4 of Office Action), Applicants agree. However, this does not mean that all L-selectin molecules cannot be used in the methods of the invention. Quite the contrary, non-cleavable L-selectins, such as those having mutated protease cleavage sites, are explicitly identified as a selectin molecule for the purposes of the invention on page 7, lines 25-27. Thus the teachings of the present invention enable one of skill in the art to modify dendritic cells to express appropriate L-selectins, to assess their expression and to test for their binding to selectin ligands.

The Examiner also stated that no guidance was provided for the use of E-selectin or P-selectin "to target dendritic cells to lymph nodes or any other tissue in vivo." Office Action at page 4. Applicants respectfully disagree. In Example 3, Applicants described expression of an E/L-selectin chimeric protein, wherein the chimeric protein contains the transmembrane and intracellular domains of L-selectin and the extracellular domain of E-selectin. Thus this protein interacts with other cells via the E-selectin extracellular domain. Example 3 demonstrates that the DCs transduced with the E/L-selectin chimera could tether and roll both *in vitro* and *in vivo* on the selectin ligand peripheral node addressin (PNAd). Therefore, Applicants explicitly taught the use of an E-selectin portion that has selectin ligand binding activity. Moreover, Applicants taught that DCs that express E-selectin selectin ligand binding domain are targeted to peripheral lymph nodes via interaction with PNAd, which is known to be expressed in peripheral lymph nodes. Additionally, the specification teaches in Example 4 that dendritic cells can bind activated platelets, which express cell surface P-selectin. It was further shown that the dendritic cell-platelet complex also bind PNAd.

The Examiner alleges that the specification "fails to teach the level of E-selectin or P-selectin expression on dendritic cells sufficient to result in accumulation of dendritic cells in peripheral lymph nodes." First, the claimed invention relates to targeting of DCs, and not necessarily to "accumulation" of DCs, as it is expected that DCs can extravasate from the blood vessel into the targeted tissue (e.g., peripheral lymph node). Second, the exact amount of selectin that needs to be expressed by the DCs for effective targeting is not required to be provided in the specification. In particular, the determination of an amount of selectin

expression that is sufficient for targeting is a matter of routine experimentation for one of ordinary skill in the art. Applicants have demonstrated that retroviral transduction of DCs with an E/L-selectin chimera is sufficient. This guidance, coupled with the knowledge of one of ordinary skill in the art, is sufficient enablement for any selectin molecules. Applicants have also provided the experimental protocol that can be used to test for the sufficiency of selectin expression with respect to *in vitro* and/or *in vivo* targeting of DCs.

The Examiner commented that receptors for E-selectin and P-selectin are not limited to peripheral lymph nodes, and that one of ordinary skill in the art could not have predicted that DCs would be targeted solely to the peripheral lymph node. Applicants assert that enablement of the claims does not depend on the selectin ligand (i.e. receptor) being expressed exclusively on peripheral lymph nodes, or the exclusive targeting of DCs to peripheral lymph node. In other words, all of the DCs need not migrate to secondary lymphoid tissue in order to meet the limitations of the claimed invention. In addition, several claims relate to the targeting of DCs to tissues other than secondary lymphoid tissue. Therefore Applicants respectfully suggest that this basis is not effective for an enablement rejection of the claims.

Regarding the Examiner's comments about the lack of teaching for the level of expression required for targeting to non-lymphoid tissue, Applicants assert that the same principles apply as for secondary lymphoid targeting and that the disclosure that was made for peripheral lymph node targeting combined with the common knowledge in the art (as admitted by the Examiner on pages 3 and 4 of the Office Action, one of ordinary skill in the art knew that certain selectin receptors/ligands were expressed on non-lymphoid tissues) is sufficient to enable one of ordinary skill in the art to practice the claimed invention throughout its scope.

The Examiner stated that no guidance was provided for the amount of dendritic cells required to induce an immune response in a mammal, or the level of immune response required for therapeutic effects. Office Action at page 6. The effect of professional antigen-presenting cells such as DCs in the induction of immune responses, including therapeutically effective immune responses, is well known in the art. Indeed, the Examiner states that this is well known on page 6 of the Office Action. The exact amounts required for such an immune response likely cannot be predicted *a priori*; instead, one of ordinary skill in the art performs routine experimentation to determine the amount of dendritic cells effective to induce an immune response with a particular combination of antigen and disease. These are matters that fall within the routine experimentation of a person of ordinary skill in the art. Applicants have provided all

of the guidance needed for one of ordinary skill in the art to set up and perform such experiments, and therefore have met the burden of enabling the claimed invention.

The Examiner stated that naïve dendritic cells are not known to produce any immune response; Applicants agree. The claimed invention, however, when reciting that an immune response will be modulated, always requires the presence of antigen. (See claims 37-49 which recite vaccine compositions and methods.) The other claims do not require the modulation of an immune response and thus properly do not require the inclusion of an antigen. Because Applicants have not presented claims that recite a therapeutic effect of a naïve dendritic cells, Applicants respectfully maintain that this portion of the enablement rejection is not warranted.

On pages 7 and 8 of the Office Action, the Examiner cites several literature references to support the proposition that "at the time of filing, targeting of vectors or cells to specific tissues or cells [sic] types in vivo was considered highly unpredictable." Applicants respectfully disagree.

The cited references relate to targeting of vectors for expression of therapeutic genes or gene products, which process the Examiner concludes is unpredictable. In contrast, Applicants invention relates to targeting of dendritic cells in accordance with a well known and understood binding reaction between selectin and selectin ligands/receptors. The binding is not an unpredictable interaction, as has been demonstrated in the art. Applicants' invention is based on the discovery that cultured dendritic cells cannot appropriately utilize the known selectin interaction to home to secondary lymphoid tissues, because the expression of functional L-selectin is reduced. Thus it is the restoration or augmentation of selectin expression in accordance with the invention that provides for dendritic cell targeting.

The references cited by the Examiner on page 7 relate to therapeutic gene therapy, not to delivery of dendritic cells to tissues that express selectin ligands. The delivery of dendritic cells is based on the discovery of a reduced expression of L-selectin in cultured DCs, and that augmentation of selectin expression by genetic modification (or treatment with activated platelets or platelet membrane microparticles) can be used to target the cells to secondary lymphoid tissues such as peripheral lymph node, or to other tissues that express appropriate selectin ligands to bind the selectin molecule expressed in the dendritic cells.

The references cited by the Examiner on page 7 relate to cancer immunotherapy, which is only one of the possible uses for the dendritic cell delivery methods of the invention, and

therefore not dispositive for enablement of the invention as a whole. Applicants also note that the quotations from these references do not suggest that *ex vivo* immunotherapy is unpredictable, and further note that the two references cited were published several years prior to the priority filing date of the instant application.

The Examiner has quoted Ross as stating that success with a single patient does not imply general utility of *ex vivo* immunotherapy. First, Ross does not state that *ex vivo* immunotherapy is unpredictable, merely that it may not be generally applicable. Second, the studies described by Ross relate primarily to the genetic modification of a patient's own cancer cells (e.g., to express certain cytokines) to enhance the immune response to the tumor cells. Third, Ross states that some of the studies provided evidence of immunological responses (first sentence of cited paragraph). Therefore, Ross does not provide any evidence that modulation of immune responses against cancer cells by delivery of dendritic cells (which again must be emphasized is only one aspect of Applicants' invention) is unpredictable.

The Examiner quoted Orkin as stating that the promise of gene therapy for cancer has not resulted in demonstrated efficacy. Orkin's comments for *ex vivo* therapy are specific to the "transfer of genes for cytokines or other immunomodulatory products to cancer cells...." Orkin at page 6, last full paragraph, emphasis added. Orkin's comments are not relevant to the methods of the invention, which as is noted above, relate in some aspects to *ex vivo* immunotherapy using dendritic cells. Applicants invention pertains in certain aspects to dendritic cells that are genetically modified to express a selectin, not a cytokine or other immunomodulatory product. Therefore, Applicants assert that Orkin is not relevant to the enablement of the claimed invention.

The Examiner appears to have considered the amount of guidance given by the specification, quantity of experimentation, and breadth of the claims. In this rejection, it appear that the Examiner did not consider the remaining *Wands* factors: 1) the nature of the invention, 2) the presence of working examples, 3) the predictability of the art, 4) the state of the prior art, and 5) the level of one of ordinary skill in the art. Applicants submit that none of these factors would weigh against a finding of enablement for the claimed invention. The nature of the invention is modification of dendritic cells (genetically or by contacting DCs with platelets or membrane microparticles) to increase selectin expression so that sufficient amounts of selectin for targeting of DCs are expressed or associated with DCs. Dendritic cells are well known to one of ordinary skill in the art, as are platelets and genetic modification of cells. In addition, working examples including the preparation of genetically modified DCs and a demonstration of their targeting to secondary lymphoid tissue were provided in the instant application (see the Examples). Further, genetic modification of cells, or contacting cells with platelets or membrane microparticles, and the testing of modified dendritic cells is routine and predictable in the art, and therefore requires only routine experimentation. The Examiner's comments with respect to the alleged unpredictability of the art were addressed above; in short, the invention does not involve gene therapy in the sense considered, and described as unpredictable (at least in 1995-6), by such references as Orkin and Ross.

With respect to the working examples *Wands* factor, the court in *In re Wright* stated that "Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples." *In re Wright* 999 F.2d 1557, 1561, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993) *citing In re Marzocchi* 439 F.2d 220, 223, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971). Applicants have provided not only broad terminology which is readily understandable to one of ordinary skill in the art, but also illustrative working examples. Thus the guidance presented is not, by itself, sufficient reason to find undue experimentation.

The last two *Wands* factors are crucial to any determination of undue experimentation. In the *Wands* case, for example, the court's decision turned on the "high level of skill in the art at the time the application was filed", and that "all of the methods needed to practice the invention were known." *Wands* at 740, 8 U.S.P.Q.2d at 1406. Applicants maintain that the

same conclusions with respect to the state of the art and the level of skill in the art are true in the instant case, and therefore must weigh heavily in favor of a finding that undue experimentation is not required.

The level of skill in the art has an important effect on the amount of guidance which must be provided to enable the invention. As the court stated in *In re Howarth*, "[i]n exchange for the patent, [the applicant] must enable others to practice his invention. An inventor need not, however, explain every detail since he is speaking to those skilled in the art." *In re Howarth*, 654 F.2d 103, 105 (C.C.P.A. 1981). Thus the level of knowledge of one of ordinary skill in the art cannot be ignored in the *Wands* factor analysis. For the standard procedures required to practice the claimed invention, the level of skill in the art is high. Applicants maintain that the person of skill in the art of molecular biology would know how to genetically modify dendritic cells to express selectins that bind selectin ligands and test such cells for the appropriate tissue targeting having in hand Applicants' disclosure.

In summary, a full analysis of the *Wands* factors strongly favors a conclusion that only routine experimentation would be required of one of ordinary skill in the art to practice the claimed invention throughout its scope.

Accordingly, in view of the amendment to the claims and the analysis above, Applicants respectfully request that the Examiner withdraw the rejections made under 35 U.S.C. §112, first paragraph.

Rejections Under 35 U.S.C. § 102(a)

The Examiner rejected claims 1, 6, 7, 28-30, 37 and 48 under 35 U.S.C. § 102(a) as anticipated by Klein et al. Applicants respectfully traverse the rejection.

The Examiner stated that the Klein reference was published on March 12, 1999. Applicants assume that this date was taken from the date written on the reference itself, "3/12/99", as provided by Applicants with an Information Disclosure Statement. This date was written on the face of the reference by the European Patent Office International Searching Authority. The European convention is to write the date in day/month/year format, not month/day/year format as is common in the United States. Therefore, Applicants assert that the "3/12/99" date refers to December 3, 1999.

This interpretation is supported by the International Search Report for the corresponding PCT application. The page of the Search Report that refers to the Klein reference is provided herewith. The entry for the Klein reference is a "X,P" reference; the "P" indicates that the priority date (which is the same as for the instant application) is earlier than the reference publication date. The entry for the Klein reference also shows that the reference was published on November 15, 1999, and presented at a meeting that occurred from December 3-7, 1999. The priority date for this application is April 1, 1999. Therefore the Klein reference is not prior art to the instant application.

Accordingly, Applicants respectfully request that the Examiner withdraw the rejection of the claims under 35 U.S.C. § 102(a).

In view of the foregoing, Applicants respectfully request that the Examiner act favorably upon the claims and issue a Notice of Allowance. If the Examiner wishes to expedite the prosecution of this application in any way, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

By: 

John R. Van Amsterdam, Reg No. 40,212
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, MA 02210
Telephone (617) 720-3500

Docket No. B00801/70170
Dated: April 2, 2002
X04/04/02

Marked-Up Claims

1.(amended) A method for delivery of dendritic cells to a secondary lymphoid tissue of a subject, comprising

providing isolated [genetically modified] dendritic cells which are genetically modified to express on the cell surface a selectin polypeptide comprising an endothelial selectin ligand binding portion of a selectin selected from the group consisting of L-selectin, E-selectin and P-selectin, and

administering the isolated genetically modified dendritic cells to the subject.

5.(amended) A method for delivery of dendritic cells to a non-lymphoid tissue of a subject where selectin ligands are expressed on endothelial cells, comprising

providing isolated [genetically modified] dendritic cells which are genetically modified to express on the cell surface a selectin polypeptide comprising an endothelial selectin ligand binding portion of a selectin selected from the group consisting of L-selectin, E-selectin and P-selectin, and

administering the isolated genetically modified dendritic cells to the subject.

28.(amended) A composition comprising isolated [genetically modified] dendritic cells which are genetically modified to express on the cell surface a selectin polypeptide comprising an endothelial selectin ligand binding portion of a selectin selected from the group consisting of L-selectin, E-selectin and P-selectin.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/08654

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KLEIN C ET AL: "Genetically engineered dendritic cells mediate increased anti-tumor immunity and can be targeted to lymph nodes." BLOOD, vol. 94, no. 10 SUPPL. 1 PART 1, 15 November 1999 (1999-11-15), page 398a XP000938706 Forty-first Annual Meeting of the American Society of Hematology;New Orleans, Louisiana, USA; December 3-7, 1999 ISSN: 0006-4971 abstract	1,2, 7-11,28, 30-34, 37,39, 40, 42-45,48
A	ROBERT C ET AL: "Targeting cultured dendritic cells to peripheral lymph nodes using a platelet bridge." JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 112, no. 4, April 1999 (1999-04), page 524 XP000861874 60th Annual Meeting of the Society for Investigative Dermatology;Chicago, Illinois, USA; May 5-9, 1999 ISSN: 0022-202X abstract	14-27
A	DIACOVO T G ET AL: "Circulating-activated platelets reconstitute lymphocyte homing and immunity in L-selecting deficient mice." BLOOD, vol. 90, no. 10 SUPPL. 1 PART 1, 15 November 1997 (1997-11-15), page 567A XP000861986 39th Annual Meeting of the American Society of Hematology;San Diego, California, USA; December 5-9, 1997 ISSN: 0006-4971 abstract	14-27